Conformation of *N*(9)-(β-D-arabinofuranosyl) adenine 5′-monophosphate (ara-AMP) in anhydrous dimethylsulphoxide monitored by ^{13}C NMR

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1. INTRODUCTION

The conformation of nucleosides and nucleotides plays an important role in their interaction with enzymes and other cellular components. This is best exemplified by the antiviral activity of 9-β-D-(arabinofuranosyl) adenine (ara-A) [1–3]. The assumption has been made [2] that this activity is due to different conformational requirements of the host-cell and viral nucleoside kinase. The aim of this work is to determine the conformation of the ara-AMP molecule as a free acid in anhydrous dimethylsulphoxide using ^3J (CH) and ^3J (CP) values.

2. MATERIALS AND METHODS

Ara-A (1 g = 3.74 mmol) from Serva (Heidelberg) was converted to ara-AMP by 0.52 ml (6 mmol) of $POCl_3$ in 10 ml $PO(OMe)_3$ during 24 h at 0°C [4]. The reaction mixture was then treated with ether, the oil-like residue dissolved in 5 ml H_2O, kept for 1 h at 0°C, then treated with acetone and ether and the precipitate dissolved in H_2O and applied to a 1.2 × 40 cm Sephadex A-25 column (130 ml, AcO^- form). The column was eluted using a linear gradient of H_2O–1 N CH_3-COOH (1 l each). The ara-AMP containing fractions (~0.5-0.7 N CH_3-COOH) were combined, evaporated to dryness in vacuo and yielded chromatographically and electrophoretically homogeneous 0.885 g (68%) ara-AMP as a white powder. The ^{13}C NMR spectra were recorded on a Bruker HFX 60 spectrometer equipped with Bruker-Data-System B-NC 12 for operation in the Fourier-transform mode.

3. RESULTS AND DISCUSSION

Assignments of the ^{13}C resonances of the heterocyclic moiety of ara-AMP (table 1) are based on the data in [5] for 5′-AMP, and proved by characteristic values of ^1J (CH) and ^3J (CH) (table 2), established for *N*(9)-adenine nucleosides [6]. Assignments of the ^{13}C signals of the carbohydrate moiety of ara-AMP (table 1) were made by using the values of ^1J (CH) [6–8] and ^2J and ^3J (CP) [9–11].

Ara-AMP was obtained as free acid and we assume it to exist as an inner salt with a monoionized phosphate group and a protonated heterocyclic base. However, in the ^{13}C NMR spectrum of ara-AMP in anhydrous dimethylsulphoxide* no appreciable shift of the ^{13}C signals of the heterocyclic base characteristic of the protonated form was observed [5,12,13].

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* In the ^{13}C NMR spectra of 5′-monophosphates of *N*(9)-(3-chloro-3-deoxy-β-D-xylofuranosyl) adenine and *N*(9)-(2-chloro-2-deoxy-β-D-arabinofuranosyl) adenine recorded in D_2O we observed the ^{13}C signal shifts characteristic of the protonated form of the base. The most populated conformational arrangement of both compounds appears to be *anti/gauche–gauche* (all *trans*) [14]. The low solubility of ara-AMP precluded the recording of the ^{13}C NMR spectrum in D_2O

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Table 1

^{13}C chemical shifts[a] $\delta_{TMS\ (internal)}$ of ara-AMP

Aglycon					Carbohydrate moiety			
C-6	C-2	C-4	C-8	C-5	C-1′	C-2′/C–3′	C-4′	C-5′
155.10	151.41	149.14	140.66	118.12	83.99	75.51/75.19	82.40	64.95

[a] Taken from a noise-decoupled Fourier-transform spectrum of 5000 transients for a saturated solution of ara-AMP in d_6-dimethylsulphoxide; spectral width 66.6 Hz/cm

The analysis of the ^3J C(4)–H(1′) and C(8)–H(1′) values (table 2) unequivocally favours a predominant *syn* population for the conformation about the *N*–glycosyl bond. From the dependence of ^3J (CH) on a dihedral angle [15,16], it can be assumed that the values of ^3J (CH) from 0°–90° should be lower than those from 90°–180°. Thus, the simultaneous measurement of the 2 values of ^3J (CH) between H–1′ and 2 ^{13}C atoms vicinal to the nitrogen atom bearing a carbohydrate fragment allowed us to make conformational assignments for the *N*-glycosyl bond. The qualitative approach used here was employed in [7,8,17,18].

The results is rather unexpected:

(i) A crystalline ara-A has an *anti* conformation [19]. From the ^1H NMR data for dilute aqueous solutions of ara-A and ara-AMP (pH ~9) an *anti* conformation has been proposed [20].

(ii) In the case of 5′-AMP the main conformation appears to be the *anti* conformation about the *N*-glycosyl bond and the *gauche–gauche* conformation about the C(4′)–C(5′) bond [18,21]. Based on the ^{13}C NMR spectroscopy data for adenosine and 5′-AMP it was suggested [22] that the 5′-phosphate group decreases the population of *syn* conformation.

The analysis of the ^3J C(4′)–P value (table 2) regarding the most populated of the 3 staggered rotamers about the C(5′)–O(5′) bond [10,11] singles out the *gauche–gauche* conformation as the most densely populated (~85%). Hence, the conformation all-*trans* (fig.1) is the most populated in the molecular fragment P–O(5′)–C(5′)–C(4′)–H(4′) of ara-AMP, as in the case of natural nucleoside 5′-monophosphates [18]. The *syn* conformation of the heterocyclic base about the glycosyl bond would

Table 2

Coupling constants of ara-AMP

^1J (CH) (Hz)[a]:	H-8	H-2	H-1′	H-2′/H-3′	H-4′	H-5′	
	214.5	201.0	165.4	149.0/149.3	156.0	147.0	
^3J (CH) (Hz)[b]:	C(8)–H(1′)		C(4)–H(1′)	C(6)–H(2)	C(4)–H(2)	C(4)–H(8)	
	1.7		4.4	10.8	13.2	<0.5	
J (CP) (Hz)[c]:	C(4′)-P		C(5′)-P				
	8.79		4.64				

[a] Taken from a proton-coupled Fourier-transform spectrum of 28 000 transients; spectral width 300 Hz/cm

[b] Taken from a proton-coupled Fourier-transform spectrum with spectral width 16.66 Hz/cm

[c] Taken from a proton-decoupled Fourier-transform spectrum with spectral width 66.6 Hz/cm

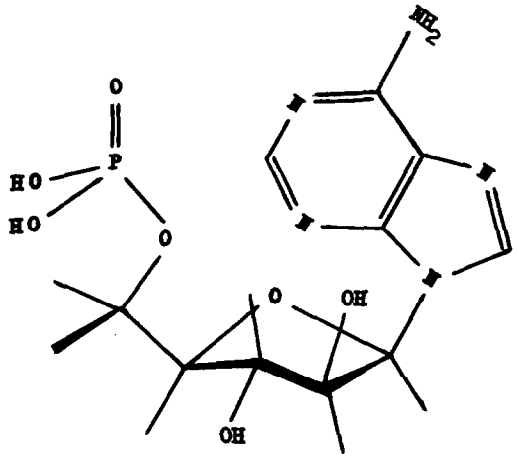

Fig.1. Structural formula and the most populated spatial arrangement of the ara-AMP molecule.

lead to a steric interaction of the latter with the phosphate group, and hence to a decrease in the population of the *gauche–gauche* conformation. However, in the case where one assumes the existence of the ara-AMP molecule in the C(4′)-*exo* or/ and C(1′)-*exo* conformations of the pentofuranose ring, then a *syn/gauche–gauche* conformational combination for the ara-AMP would seem quite probable. In this respect two facts are worth mentioning:

(i) A higher conformational mobility of the furanose ring is assumed for *arabino* nucleosides as distinct from ribofuranosides [19,23,24];

(ii) A theoretical conformational analysis of the ara-A molecule revealed the energy minimum for positioning the heterocyclic base in the *syn* region with a torsion angle value χ of 190–250° [3].

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